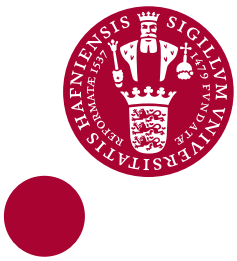

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Identification of a PVL-negative SCC*mec*-IVa sublineage of the methicillin-resistant *Staphylococcus aureus* CC80 lineage: understanding the clonal origin of CA-MRSA

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ABSTRACT

Objectives: Community-acquired (CA) methicillin-resistant *Staphylococcus aureus* (MRSA) isolates belonging to clonal complex 80 (CC80) are recognized as the European CA-MRSA. The prevailing European CA-MRSA clone carries a type IVc staphylococcal cassette chromosome *mec* (SCC*mec*) and expresses Panton-Valentine leukocidin (PVL). Recently, a significant increase of PVL-negative CC80 MRSA has been observed in Denmark. The aim of this study was to examine their genetics and epidemiology, and to compare them to the European CA-MRSA clone in order to understand the emergence of PVL-negative CC80 MRSA.

Methods: Phylogenetic analysis of the CC80 *S. aureus* lineage was conducted from whole-genome sequences of 217 isolates (23 methicillin-susceptible *S. aureus* and 194 MRSA) from 22 countries. All isolates were further genetically characterized in regard to resistance determinants and PVL carriage, and epidemiologic data were obtained for selected isolates.

Results: Phylogenetic analysis revealed the existence of three distinct clades of the CC80 lineage: (a) an methicillin-susceptible *S. aureus* clade encompassing Sub-Saharan African isolates ($n = 13$); (b) a derived clade encompassing the European CA-MRSA SCC*mec*-IVc clone ($n = 185$); and (c) a novel and genetically distinct clade encompassing MRSA SCC*mec*-IVa isolates ($n = 19$). All isolates in the novel clade were PVL negative, but carried remnant parts (8–12 kb) of the PVL-encoding prophage ΦSa2 and were susceptible to fusidic acid and kanamycin/amikacin. Geospatial mapping could link these isolates to regions in the Middle East, Asia and South Pacific.

Conclusions: This study reports the emergence of a novel CC80 CA-MRSA sublineage, showing that the CC80 lineage is more diverse than previously assumed. **S.M. Edslev, Clin Microbiol Infect 2018;24:273**

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) appeared in the early 1960s in hospital-associated infections, but from the 1990s a global emergence of community-acquired (CA) MRSA infections among healthy people was observed [1–4]. Today, several genetic lineages of CA-MRSA are present worldwide, with specific lineages predominating distinct geographic regions [4–9]. The clonal complex 80 (CC80) lineage is known as the European CA-MRSA clone despite its high prevalence both in the Middle East and Northern Africa [6,10]. The prevailing CC80 MRSA clone expresses the Panton-Valentine leukocidin (PVL) toxin encoded by the *lukS/F*-PV genes in the ΦSa2 prophage and contains a type IVc

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staphylococcal cassette chromosome *mec* (SCC*mec*) element. Furthermore, strains belonging to the clone are generically resistant to tetracycline, fusidic acid and aminoglycosides (kanamycin/amikacin) [5,10–14]. We recently delineated the evolutionary history of the European CC80 CA-MRSA clone, which suggested that the origin of the clone was a PVL-positive, methicillin- and fusidic acid–susceptible ancestral lineage in Sub-Saharan Africa [10]. The data indicated a single acquisition of an SCC*mec*-IVc carrying the kanamycin/amikacin resistance genes (*aadK* and *aphA*) and a plasmid encoding fusidic acid resistance (*fusB*) during or upon its dissemination out of this region. Upon acquisition of these elements, the clone spread rapidly across Northern Africa, the Middle East and Europe to become the first dominant CA-MRSA clone in these regions.

In the last few years, an increased number of PVL-negative CC80 CA-MRSA isolates has been observed in Denmark, a genotype that we previously found to be rare [3]. The aim of this study was to examine the genetics and epidemiology of these PVL-negative CC80 MRSA isolates, and to compare them to the European CA-MRSA clone in order to understand their emergence.

Methods

Bacterial isolates

The present study included 217 CC80 *S. aureus* isolates (23 methicillin-susceptible *S. aureus* (MSSA) and 194 MRSA) originating from four different data sets. First, we included all CC80 MRSA strains that had been isolated in the capital region of Denmark between 2013 and 2015 ($n = 107$), as hospitals here have genome sequenced all MRSA isolates from 2013 and onwards. Additionally, all PVL-negative CC80 MRSA isolated between 2008 and 2015 identified in the Danish national MRSA surveillance register were included ($n = 10$). Furthermore, three out of four reported PVL-negative and SCC*mec*-IVa-positive CC80 MRSA isolates from Bangladesh were included [15]. A literature search was performed to ensure that no other published PVL-negative and SCC*mec*-IVa-positive CC80 MRSA isolates were overlooked. Lastly, we included 97 isolates (23 MSSA and 74 MRSA) from our previous European CA-MRSA study [10]. These isolates were sampled between the years 1993 and 2010 in Europe, North Africa, Sub-Saharan Africa, the Middle East and Asia. Only one isolate from each individual was included.

Clinical and epidemiologic information

Clinical and epidemiologic data were obtained from both previously published studies [10,15] and the Danish national MRSA surveillance register (Danish Data Protection Agency protocol 2001-14-0021). The following information was obtained for all cases: country of origin and year of isolation. For all isolates clustering in the novel clade of CC80, additional clinical and epidemiologic information was extracted: sex, age, ethnicity, family relations between affected subjects, source of MRSA acquisition (e.g. imported, household, or hospital related) and whether the MRSA was related to infection or asymptomatic carriage.

Genome sequencing

Sequence data from the 97 isolates in the fourth data set were available at the European Nucleotide Archive (ENA; http://www.ebi.ac.uk/ena, accession number PRJEB6777). Paired-end sequences (2×250 or 2×150) for the remaining 120 CC80 isolates were obtained (MiSeq; Illumina, San Diego, CA, USA). The sequence data are available at ENA (accession number PREJ19874).

Phylogenetic analysis

Single nucleotide polymorphisms (SNPs) in the core genome were detected using the Northern Arizona SNP Pipeline (NASP; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5320593/) by aligning Illumina reads against *S. aureus* 11819-97, a Danish CC80 CA-MRSA isolate (GenBank accession number NC_017351) [16]. The presence of recombination was investigated using the pairwise homoplasy index test implemented in SplitsTree v4.13.1 [17]. Genome-wide phylogenetic relationships were reconstructed using the maximum-likelihood algorithm implemented in PhyML v3.412 [18], using Smart Model Selection with the Bayesian Information Criterion with 100 bootstrap replicates with nearest neighbour interchange and subtree priming and grafting searches for optimal tree structures. The phylogenies were rooted with the community-associated CC1 strain MW2 (GenBank accession number BA000033). iTOL (http://itol.embl.de/) was used for visualization of the phylogenetic tree.

Time of divergence analyses

Coalescence-based analyses were used to investigate the timing of most recent common ancestor (TMRCA) using BEAST v1.7.5 software (http://beast.bio.ed.ac.uk/) [19]. Evaluation of the models, which included the general-time reversible and the Hasegawa-Kishino-Yano substitution models, as well as both strict and relaxed molecular clock under different demographic models (Bayesian skyline, constant population and exponentially growing population tree priors), was performed by Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/). For each analysis, 200 million steps were performed, with sampling every 10 000th generation. The first 10% was discarded as burn-in.

Genetic typing and detection of virulence factors

Isolates were *spa* typed using Sanger sequencing as described previously [20,21]. Whole-genome sequences were *de novo* assembled using CLCbio Genomics Workbench v8.5.1 (Qiagen, Aarhus, Denmark), and contigs were used for *in silico* multilocus sequence typing (MLST) using MLST v1.8 (https://cge.cbs.dtu.dk/services/MLST/) [22]. Resistance-associated mutations in *fusA* and the presence of various resistance genes (e.g. *fusB, tet*(K) and *aadK*/*aphA*) were identified *in silico*. SCC*mec* elements were identified and subtyped *in silico* by comparison of CC80 MRSA cassettes to available reference sequences for SCC*mec* type IVa as previously described [10]. SNPs in the accessory gene regulator receptor C gene (*agrC*) were identified in all samples using *agrC* from strain 11819-97 (NC_017351) as reference. Sequences were analysed for the presence of the PVL-encoding genes *lukS*/*F*-PV and the associated ΦSa2 prophage using the variant found in strain 11819-97 as a reference. The genetic diversity of ΦSa2 prophage and the remnant parts of ΦSa2 identified in this study were examined using NASP and included reference genomes from five distinct genetic lineages of *S. aureus* as described previously [10].

Results

This study included 217 CC80 *S. aureus* isolates (23 MSSA and 194 MRSA) sampled between 1993 and 2015 in 22 countries; 146 isolates were collected in Denmark.

Five MLST types were observed among the isolates, with the majority belonging to sequence type (ST) 80 ($n = 210$; 97%) and the remaining being single-locus variants of ST80. Seventeen different *spa* types were identified, with t044 being the most frequent ($n = 138$; 64%) (Supplementary Table S1).

Phylogenetic relationship of CC80 complex

The isolates were sequenced to a depth of >20× coverage. A total of 4880 SNPs were identified within the conserved core genome (57% of the reference chromosome). Twelve SNPs were removed as a result of putative recombination events, after which no statistical significant evidence of recombination was identified (p 0.4336). The phylogenetic analyses revealed the existence of three distinct clades in the CC80 complex (Fig. 1). One clade comprised 13 MSSA isolates with strong links to Sub-Saharan African countries, a second clade defining the European CA-MRSA clone comprised 185 isolates (10 MSSA and 175 MRSA) and a third and novel MRSA clade included 19 CC80 MRSA isolates (three Bangladeshi ST1931 isolates and 16 Danish ST80 isolates). *spa* types of the isolates in the three distinct clades revealed overlap, though several *spa* types were unique to specific clades.

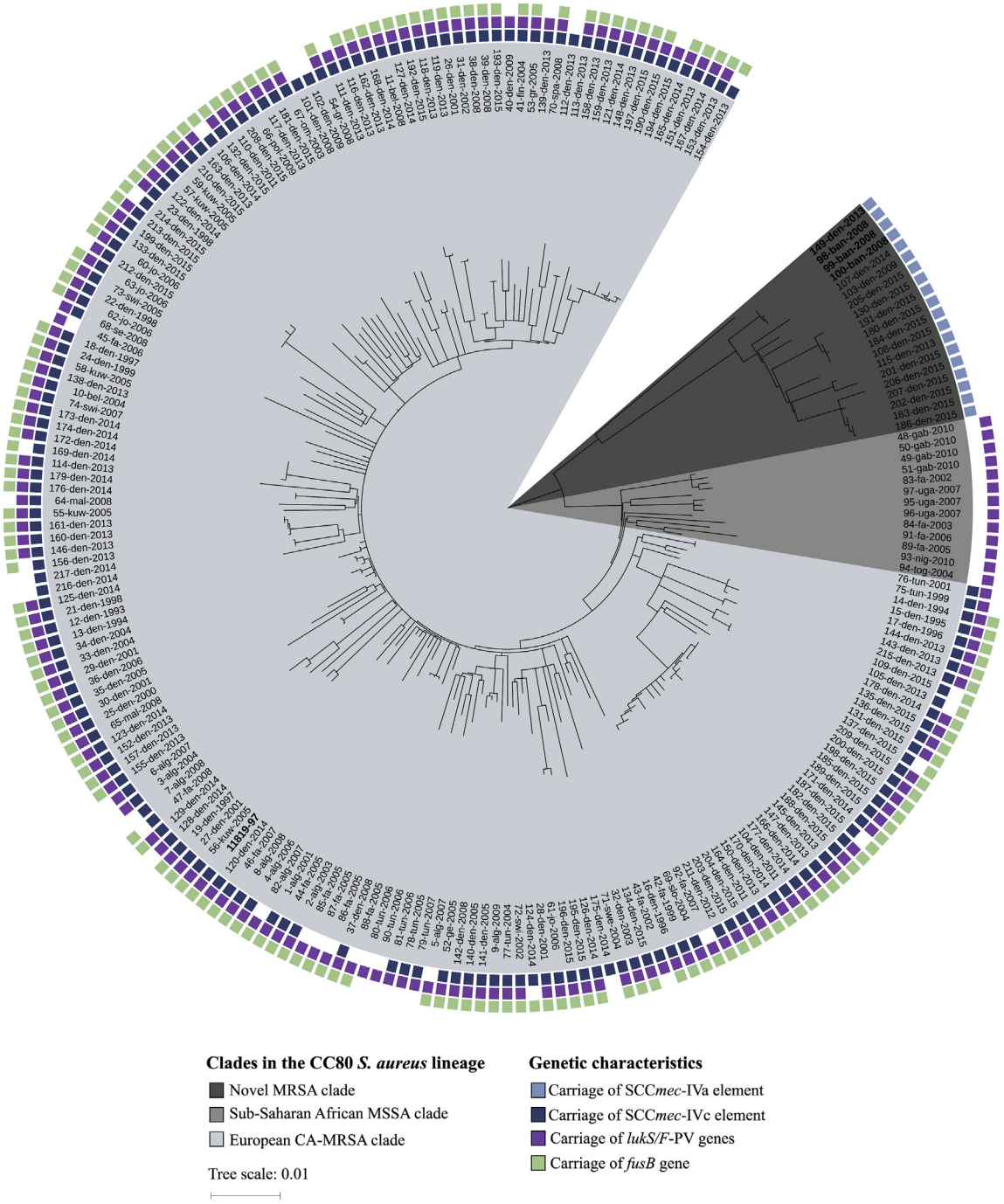

Fig. 1. Maximum likelihood phylogeny of CC80 *Staphylococcus aureus* lineage. Phylogeny is based on 4868 single-nucleotide polymorphisms in core genome and reveals three distinct clades of lineage: Sub-Saharan African MSSA clade, European CA-MRSA clade and novel MSRA clade. Clades are marked with shades of grey. Information on selected genetic markers includes: carriage of SCC*mec* type IVa (light blue) or IVc (dark blue), carriage of PVL-encoding genes *lukS*/*F*-PV (purple) and carriage of *fusB* (green). Isolate IDs referred to in text are bold. CA, community acquired; CC, clonal complex; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus;* PVL, Panton-Valentine leukocidin; SCC*mec*, staphylococcal cassette chromosome *mec*.

A Bayesian analysis (Supplementary Fig. S1) revealed that the TMRCA of the isolates in the Sub-Saharan African MSSA and the European CA-MRSA clades were dated to 1977 (95% highest posterior densities (HPDs), 1946–2023) and 1984 (95% HPDs, 1957–2019), respectively. Eighteen of 19 isolates in the novel MRSA clade were found to be closely related and formed a distinct cluster with a TMRCA estimated to be in 2000 (95% HPDs, 1990–2022). The remaining isolate, 149-den-2013, formed a deep branch distant from the other 18 isolates; similar topologies were observed in both the Bayesian and maximum-likelihood derived analyses (Fig. 1). The TMRCA for the CC80 lineage was estimated to 1942 (95% HPDs, 1901–2054).

Epidemiologic description of novel MRSA clade

The three ST1931 isolates from Bangladesh were all isolated from patients with diabetes at one hospital in 2008 and clustered closely together in the phylogeny with only two SNPs separating them, thus supporting local transmission. Clinical and demographic data were available for most of the Danish isolates clustering in the novel MRSA clade, where a single isolate was isolated in 2009 and the remaining 15 were isolated between 2013 and 2015 (Table 1). Nine of the isolates were linked to patients from three different families and showed close intrafamily clustering (0–20 SNPs). The epidemiologic data suggested ten unrelated introductions. Seven of these were related to foreign ethnicity and/or import from countries in the Middle East, Asia or South Pacific. Twelve of the 19 MRSA isolates were related to infection, with 11 of these being skin and soft tissue infections, six were related to asymptomatic carriage and the source of the last isolate was not known (Table 1).

SCCmec identification

All MRSA isolates in the European CA-MRSA clade ($n = 175$) carried a type IVc SCC*mec* element. The majority (75%, 132/175) of these contained the aminoglycosides resistance genes *aadK* (*ant(6)-Ia*) and *aphA* (*aph(3′)-III*), integrated in the J2 region. All 19 MRSA isolates from the novel MRSA clade carried a type IVa SCC*mec*. With the exception of one isolate (149-den-2013), all SCC*mec*-IVa were largely identical both in size (26 kb) and genetic content. A 1.9 kb long transposon in region J1, adjacent to the *ccr* complex, was identified in all SCC*mec*-IVa cassettes, with the exception of isolate 149-den-2013.

Identification of PVL genes

All MSSA isolates in the Sub-Saharan African MSSA clade and the majority ($n = 167$; 90%) of the isolates in the European CA-MRSA clade contained the *lukS/F*-PV genes, whereas none of the isolates clustering in the novel MRSA clade contained these. However, all of these isolates carried remnants of the PVL-carrying prophage ΦSa2, i.e. between 8 and 12 kb of the full-length ΦSa2 (58 kb). Similarly, the PVL-negative isolates in the European CA-MRSA clade also carried various remnant prophage parts. In all PVL-negative isolates, these were incorporated in the same chromosomal position as ΦSa2 in the CC80 reference strain. Investigating the genetic diversity of these remnant parts with the remaining CC80 isolates and other ΦSa2-positive CA-MRSA lineages (Supplementary Fig. S2) confirmed a distinct ΦSa2-CC80 cluster with only a single SNP observed within the CC80 cluster, which strongly indicated a single acquisition of the ΦSa2 prophage into an ancestral CC80 MSSA background more than 70 years ago.

Identification of antibiotic resistance genes

The prevalence of the trademark CC80 antibiotic resistance genes (*mecA*, *fusB*, *tet*(K) and *aadK/aphA*) in the three clades is listed in Table 2. The fusidic acid resistance gene *fusB* and kanamycin/amikacin resistance genes *aadK/aphA* were identified in 159 and 133 of the isolates (73% and 61%), respectively, all clustering in the

Table 2
Prevalence of antibiotic resistance genes

Gene	*n* (%) for:			
	All isolates ($n = 217$)	Sub-Saharan African MSSA clade ($n = 13$)	European CA-MRSA clade ($n = 185$)	Novel MRSA clade ($n = 19$)
mecA	194 (89%)	0	175 (95%)	19 (100%)
fusB	159 (73%)	0	159 (86%)	0
tet(K)	153 (71%)	7 (54%)	143 (77%)	3 (16%)
aadK/aphA	133 (61%)	0	133 (72%)	0

aadK/aphA, kanamycin/amikacin resistance; CA, community acquired; *fusB*, fusidic acid resistance; *mecA*, methicillin resistance; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*; *tet*(K), tetracycline resistance.

Table 1
Clinical and demographic data related to isolates clustering in the novel CC80 PVL-negative CA-MRSA clade

Isolate[a]	MLST	Country of isolation	Sex	Birth year	Family relation[b]	Acquisition	Ethnicity	Infection/carriage
98-ban-2008	ST1931	Bangladesh	M	1967		Hospital	Bangladeshi	SSTI
99-ban-2008	ST1931	Bangladesh	F	2000		Hospital	Bangladeshi	SSTI
100-ban-2008	ST1931	Bangladesh	M	1976		Hospital	Bangladeshi	SSTI
103-den-2009	ST80	Denmark	F	1983		Unknown	Danish	Unknown
107-den-2014	ST80	Denmark	F	2005		Import (Bangladesh)	Burmese	SSTI
108-den-2015	ST80	Denmark	F	1996		Import (Asia—unknown country)	Danish	SSTI
115-den-2013	ST80	Denmark	F	2006		Import (Dubai)	Danish	SSTI
149-den-2013	ST80	Denmark	M	1951		Import (Philippines)	Philippine	SSTI
130-den-2015	ST80	Denmark	F	1989	1	Household	Afghani	Carriage
180-den-2015	ST80	Denmark	F	1988	1	Import (Unknown country)	Afghani	Other infection
184-den-2015	ST80	Denmark	F	2015	1	Household	Afghani	SSTI
191-den-2015	ST80	Denmark	M	1987	1	Household	Afghani	Carriage
183-den-2015	ST80	Denmark	F	1987	2	Import (New Zealand)	Danish	SSTI
186-den-2015	ST80	Denmark	M	1982	2	Household	Danish	Carriage
201-den-2015	ST80	Denmark	F	1939		Unknown	Danish	SSTI
202-den-2015	ST80	Denmark	M	1984	3	Household	Danish	SSTI
206-den-2015	ST80	Denmark	F	2015	3	Household	Danish	Carriage
207-den-2015	ST80	Denmark	F	1987	3	Household	Danish	Carriage
205-den-2015	ST80	Denmark	M	2014		Import (Jordan)	Danish	Carriage

CC80, clonal complex 80; MLST, multilocus sequence typing; SSTI, skin and soft tissue infection; ST, sequence type.
[a] Each isolate is from an individual person.
[b] Nine isolates could be linked to three distinct families, designated 1, 2 and 3.

European CA-MRSA clade. Three isolates in the European CA-MRSA clade were fusidic acid resistant due to specific *fusA* mutations (Supplementary Table S1). A total of 153 isolates (71%) carried the *tet*(K) gene (tetracycline resistance) encoded on plasmid pT49 or pT181. The gene was present in isolates in all three clades, with the highest prevalence in the European CA-MRSA clade.

Discussion

In this study, we have shown that a novel sublineage of CC80 CA-MRSA has been introduced into the capital region of Denmark within the last few years. This novel sublineage differs from the typical European CA-MRSA clone not only by its genetic and antimicrobial susceptibility characteristics (PVL negative, carriage of an SCC*mec*-IVa element and susceptibility to fusidic acid and aminoglycosides) but also by its unique phylogenetic clustering. Thus, these results add a new chapter to the evolutionary history of the CC80 lineage.

Although all isolates in the novel MRSA clade were PVL negative, this cannot be used as a reliable marker, as several isolates in the European CA-MRSA clade ($n = 18$; 10%) lacked PVL. In fact, during the last few years, there has been an increase in PVL-negative CC80 MRSA strains in Denmark. Whereas none of the tested ($n = 119$) CC80 MRSA strains submitted to the Danish National MRSA surveillance laboratory between 1999 and 2006 ($n = 418$) were PVL negative, 18% (36/202) of all CC80 strains submitted between 2013 and 2015 were PVL negative (data not shown). PVL-negative CC80 MRSA strains have also been reported in other countries, where this clone is endemic [14].

All PVL-negative isolates in this study carried remnants of prophage ΦSa2, indicating that they have derived from a PVL-positive ancestor that, during evolution, lost part of the phage, including the *lukS*/*F*-PV genes. Our results suggest that multiple independent losses must have occurred in the CC80 lineage, as multiple ΦSa2 remnants variants were identified and PVL-negative isolates in the European CA-MRSA clade occurred sporadically across the phylogeny (Fig. 1). The rise in clinical cases of PVL-negative CC80 MRSA supports that PVL is not an essential genetic trait associated with the success of the clone, at least nowadays. A nonsynonymous *agrC* SNP (L184I) was one of four unique SNPs described to distinguish the European CA-MRSA clade from the Sub-Saharan African MSSA clade [10]. All isolates from the novel clade contained the same variant (184L) as the Sub-Saharan African isolates (data not shown).

Analysis of the SCC*mec* revealed that different type IV elements have been introduced into the CC80 lineage, with SCC*mec*-IVc exclusively associated with the European CA-MRSA clone and SCC*mec*-IVa in the novel MRSA clade. It is likely that SCC*mec*-IVa elements were introduced into the lineage twice, as one of the isolates in the novel clade (isolate 149-den-2013) formed a long, deep branch (Fig. 1) and carried a slightly different variant of SCC*mec*-IVa that lacked a transposable element in J1. If the SCCmec-IVa only were introduced ones back in the late-1940s (Supplementary Fig. S1) and thus precede the emergences of SCC*mec* type IV by decades [10], this would change the history of SCC*mec* considerably [23].

The MRSA isolates in the novel clade also differed from the European CA-MRSA by being susceptible to both fusidic acid and aminoglycosides (Table 2).

Thirteen (68%) of the 19 isolates in the novel MRSA clade were linked to countries in the Middle East, Asia or the South Pacific by recent travel and/or foreign ethnicity (Table 1).

A limitation of the present study is that the majority of the isolates in the novel clade are from Denmark, as reports of CC80 MRSA with the genotypic characteristics presented here are limited [15]. Additional investigations on the presence and prevalence of this subtype in the Middle East, Asia and the South Pacific are therefore necessary in order to validate this link. In addition, identification and geographic knowledge of MSSA isolates basal to the novel MRSA clade would be important for the determination of a geographical origin of the CC80 lineage.

In summary, we here describe the presence of a novel PVL-negative SCC*mec*-IVa CC80 MRSA clone in Denmark, but with the mobility of people both regionally and internationally, there is certainly an opportunity for this clone to have disseminated to other geographic regions. Our results highlight the usefulness of routine surveillance of MRSA using next-generation sequencing combined with detailed bioinformatic analysis to further understand population dynamics and emergence of novel lineages.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2017.06.022.

Transparency declaration

All authors report no conflicts of interest relevant to this article.

References

[1] Chambers HF, Deleo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat Rev Microbiol 2009;7:629–41.
[2] Klein E, Smith DL, Laxminarayan R. Community-associated methicillin-resistant *Staphylococcus aureus* in outpatients, United States, 1999–2006. Emerg Infect Dis 2009;15:1925–30.
[3] Larsen AR, Stegger M, Bocher S, Sorum M, Monnet DL, Skov RL. Emergence and characterization of community-associated methicillin-resistant *Staphyloccocus aureus* infections in Denmark, 1999 to 2006. J Clin Microbiol 2009;47:73–8.
[4] David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 2010;23:616–87.
[5] Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 2003;9:978–84.
[6] Mediavilla JR, Chen L, Mathema B, Kreiswirth BN. Global epidemiology of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA). Curr Opin Microbiol 2012;15:588–95.
[7] Chen CJ, Huang YC. New epidemiology of *Staphylococcus aureus* infection in Asia. Clin Microbiol Infect 2014;20:605–23.
[8] Williamson DA, Coombs GW, Nimmo GR. *Staphylococcus aureus* 'Down Under': contemporary epidemiology of *S. aureus* in Australia, New Zealand, and the South West Pacific. Clin Microbiol Infect 2014;20:597–604.
[9] Carrel M, Perencevich EN, David MZ. USA300 methicillin-resistant *Staphylococcus aureus*, United States, 2000–2013. Emerg Infect Dis 2015;21:1973–80.
[10] Stegger M, Wirth T, Andersen PS, Skov RL, De Grassi A, Simoes PM, et al. Origin and evolution of European community-acquired methicillin-resistant *Staphylococcus aureus*. MBio 2014;5:e01044-14.
[11] Tristan A, Bes M, Meugnier H, Lina G, Bozdogan B, Courvalin P, et al. Global distribution of Panton-Valentine leukocidin–positive methicillin-resistant *Staphylococcus aureus*, 2006. Emerg Infect Dis 2007;13:594–600.
[12] Larsen AR, Bocher S, Stegger M, Goering R, Pallesen LV, Skov R. Epidemiology of European community-associated methicillin-resistant *Staphylococcus aureus* clonal complex 80 type IV strains isolated in Denmark from 1993 to 2004. J Clin Microbiol 2008;46:62–8.
[13] Goering RV, Shawar RM, Scangarella NE, O'Hara FP, Amrine-Madsen H, West JM, et al. Molecular epidemiology of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates from global clinical trials. J Clin Microbiol 2008;46:2842–7.
[14] Harastani HH, Tokajian ST. Community-associated methicillin-resistant *Staphylococcus aureus* clonal complex 80 type IV (CC80-MRSA-IV) isolated from the Middle East: a heterogeneous expanding clonal lineage. PLoS One 2014;9:e103715.

[15] Paul SK, Ghosh S, Kawaguchiya M, Urushibara N, Hossain MA, Ahmed S, et al. Detection and genetic characterization of PVL-positive ST8-MRSA-IVa and exfoliative toxin D–positive European CA-MRSA-Like ST1931 (CC80) MRSA-IVa strains in Bangladesh. Microb Drug Resist 2014;20:325–36.
[16] Stegger M, Price LB, Larsen AR, Gillece JD, Waters AE, Skov R, et al. Genome sequence of *Staphylococcus aureus* strain 11819-97, an ST80-IV European community-acquired methicillin-resistant isolate. J Bacteriol 2012;194:1625–6.
[17] Huson DH, Bryant D. Application of phylogenetic networks in evolutionary studies. Mol Biol Evol 2006;23:254–67.
[18] Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 2003;52:696–704.
[19] Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol 2012;29:1969–73.
[20] Larsen AR, Stegger M, Sorum M. *spa* typing directly from a *mecA, spa* and *pvl* multiplex PCR assay—a cost-effective improvement for methicillin-resistant *Staphylococcus aureus* surveillance. Clin Microbiol Infect 2008;14:611–4.
[21] Bartels MD, Petersen A, Worning P, Nielsen JB, Larner-Svensson H, Johansen HK, et al. Comparing whole-genome sequencing with Sanger sequencing for *spa* typing of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 2014;52:4305–8.
[22] Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, et al. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 2012;50:1355–61.
[23] Harkins CP, Pichon B, Doumith M, Parkhill J, Westh H, Tomasz A, et al. Methicillin-resistant Staphylococcus aureus emerged long before the introduction of methicillin into clinical practice. Genom Biol 2017;18:130.